Hematotoxic and Hepatotoxic Effects of Dichlorvos at Sublethal Dosages in Rats

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ABSTRACT: The present study was designed to understand the effects of sublethal concentrations of dichlorvos (DIC) on hematological constituent [red blood corpuscles, white blood corpuscles (WBC), mean cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet counts, hemoglobin and hematocrite levels] and serum damage marker enzymes (aspartate aminotransferase, alanin aminotransferase, alkaline phosphatase, and lactate dehydrogenase) in rats at subacute period under laboratory conditions. DIC at dosages of 5 and 10 ppm was administered orally to six male rats *ad libitum* during the tests for 4 weeks consecutively. According to the results, DIC treatments increased significantly the levels of serum marker enzyme activities, whereas they did not change hematologic constituent except for WBC number treated with both dosages of DIC. The observations presented led us to conclude that the administrations of subacute DIC induced the levels of damage marker enzymes and leukocytosis. © 2008 Wiley Periodicals, Inc. Environ Toxicol 24: 128–132, 2009.

Keywords: dichlorvos; hematological constituents; serum marker enzymes; rat

INTRODUCTION

Dichlorvos (DIC) is an organophosphate (OP) compound used to control household, public health, and stored product insects. It is effective against mushroom flies, aphids, spider mites, caterpillars, thrips, and white flies in greenhouse, outdoor fruit, and vegetable crops. DIC is used to treat a variety of parasitic worm infections in dogs, livestock, and humans. DIC can be fed to livestock to control botfly larvae in the manure. It acts against insects as both contact and stomach poison. It has been used to make pet collars and pest strips (Dikshith, 1991; Matsumura, 1985; Yarsan and Cakir, 2006). The uncontrolled use of these insecticides in agriculture and public health operation has increased the scope of ecological imbalance and thus many nontarget organisms have become victims (Das and Mukherjee, 2000). In the literature, it is reported that OPIs are neurotoxic in nature by acting as inhibitors of neuronal cholinesterase (ChE) activity (Ecobichon, 1996). However, some studies reported that OPIs caused lipid peroxidation (Dasgupta et al., 1992; Bagchi et al., 1995; Gultekin et al., 2000) in vertebrates. When feeded with DIC, studies indicate that dosages of DIC very much larger than dosages that inhibit ChE are needed to produce illness (Roberson and Nolan, 1988; Thienes and Haley, 1972). Campbell and Ofurum (1986) investigated the effects of DIC on serum and liver enzyme [glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT)] changes in rats after short-term exposure to DIC. Their study results indicated that DIC significantly elevated 2–6 h after i.p. injections of single dosages (2.5 mg/kg body wt) of DIC. Also, GOT and GPT activities are significantly increased in serum and liver of rats receiving daily injections (0.25 mg/kg body wt) for 7 days.

DIC is rapidly absorbed and degraded in all mammalian species (FAO/WHO, 1993). DIC metabolism after different routes of administration in man, rat, mouse, swine, and

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hamsters has been reported to be rapid, with similar metabolites in all species (Blair et al., 1975; Hutson et al., 1971a,b; Hutson and Hoadley, 1972a,b; Page et al., 1971, 1972). DIC degradation follows two main pathways. It is hydrolyzed to dimethylphosphate and dichloroacetaldehyde in the plasma and liver. The vinyl-phosphate bond of DIC is rapidly cleaved as the first step in degradation. A smaller fraction of DIC can be converted to desmethyl-DIC by the glutathione-dependent enzymatic system, which breaks down to methylphosphate and dichloroacetaldehyde. Intermediate metabolites that have been identified in tissues soon after exposure are dimethyl phosphate, dichloroacetaldehyde, dichloroethanol, dichloroacetic acid, and desmethyl-DIC (Page et al., 1972). There is no evidence of accumulation of DIC or its metabolites in the animal blood or tissues (WHO, 1989). Urine and respired air are major routes for elimination in man and other mammals (Hutson and Hoadley, 1972a).

DIC is one of the most widely used OPIs in agriculture and public health programs. DIC is also one of the most used OPIs in the region of Van, Turkey. The aims of the current study were to evaluate the effects of subacute exposure of DIC on serum marker enzymes and hematological constituent in rats. For this aim, the treatment of DIC was done orally, because the effect of chemicals represent a well-characterized *in vivo* toxicity model system and this evaluation was done by determining serum liver damage marker enzymes and hematological parameters.

MATERIALS AND METHODS

Materials

DIC used in experimentation was obtained from Agricultural Struggle Center, Van, Turkey. This stock solution was appropriately diluted with the test water to achieve the desired concentrations of DIC.

Animals

Rats (Sprague–Dawley albino) weighing 150–200 g were provided by the animal house of the Sciences Faculty of Yuzuncu Yil University, and were housed in three groups, each group containing six rats. All animals were fed a group wheat–soybean–meal-based diet and water *ad libitum* in stainless cages, and received humane care according to the criteria outlined in the ‘Guide for the Care and Use of Laboratory Animals’ prepared by the National Academy of Science and published by the National Institutes of Health. The animals were housed at 20 ± 2°C in a daily light/dark cycle.

Treatment of Chemical

This investigation was performed on male rats. DIC at dosages of 5 and 10 ppm was used. Rats were exposed DIC *ad libitum* during the tests for 4 weeks. Control rats were given only distilled water. Daily water consumption of rats was 28 ± 2 mL during the tests.

At the end of the treatment, rats were anesthetized by inhalation of diethyl ether, and they were then sacrificed. Blood samples were obtained from the cardiac puncture by using syringe for the determination of hematological and biochemical constituent. Blood samples were drawn immediately into ice-chilled siliconized disposable glass tubes. The serum samples were obtained by centrifuging blood samples at 3000 rpm for 15 min at 4°C, and serum marker enzyme activities were measured in these serum samples. For hematological constituents, the blood samples were drawn using EDTA as an anticoagulant.

Measurement of Enzyme Levels

Serum enzyme activities [aspartate aminotransferase (AST) (EC 2.6.1.1), alanin aminotransferase (ALT) (E.C 2.6.1.2), lactate dehydrogenase (LDH) (EC 1.1.1.27), and alkaline phosphatase (ALP) (EC 3.1.3.1)] were measured by an auto analyzer (BM/HITACHI-911), using the kits (DPC; Diagnostic Products Corporation, USA).

Hematological Study

Freshly collected blood samples were analyzed for hematological assay using an automatic hematological assay analyzer (Swelab 24D). Different tested hematological parameters were as follows: red blood corpuscles (RBC), white blood corpuscles (WBC), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), MCH concentration (MCHC), platelet (PLT) counts, hemoglobin (Hb) and hematocrite (HCT) levels.

Analysis of Data

The statistical analyses were made using the minitab 13 for windows packet program. Means and standard deviations were calculated according to the standard methods for all parameters. One-way ANOVA statistical test was used to determine the differences between means of the treatments and the control group accepting the significance level at $P \leq 0.05$.

RESULTS

Following the exposure of 5 and 10 ppm dosages of DIC, the body weight of rats were found to reduce slightly. The effects of DIC administration on liver damages index were evaluated as damage marker enzymes in serum samples from control and treated rats. The results showed that DIC caused an increase in AST, ALT, LDH, and ALP activities

TABLE I. Effects of DIC on serum marker enzyme activities and body weight of rat

Parameters	Control (Mean ± SD)	5 ppm DIC (Mean ± SD)	10 ppm DIC (Mean ± SD)
Body weight (g)			
Beginning	176 ± 17	164 ± 12	168 ± 20
Finally	187 ± 14	160 ± 17	162 ± 19
AST (U/L)	164.5 ± 11.7	211.1 ± 30.2*	212.7 ± 27.8*
ALT (U/L)	37.5 ± 1.4	45.2 ± 4.1*	45.8 ± 2.7*
LDH (U/L)	3337.3 ± 418.7	4626.8 ± 844.1*	4464.3 ± 724.7*
ALP (U/L)	136.8 ± 15.9	141.3 ± 20.8*	233.5 ± 16.9*

* Significantly different from control rats at $P \leq 0.05$ (One-way ANOVA).

in rats exposed to 5 and 10 ppm dosages of DIC (Table I). With regard to the hematotoxicity of DIC subacute exposure, no alteration of the hematological values was observed in rats treated with both dosages of DIC except for the WBC number (Table II).

DISCUSSION

The effects of pollutants on nature became a field of interest for scientists from the beginning of the second half of the 20th century, and subsequently investigation on effect of these pollutants on human beings, plants, and animals were initiated. DIC is widely used throughout the world as a wide-spectrum insecticide for numerous harmful agricultural crops. Also, it has widely and effectively been used throughout the world with applications in agriculture and horticulture for controlling insects in crops. Although DIC is well known to have general health effects, little is known as regards to which extent the chemical is hematotoxic and hepatotoxic on vertebrata.

In the present study, experimental group was exposed to two dosage of the chemical substance. Liver enzymes and hematologic constituents were used as important biomarkers for detection of hepatotoxic and hematotoxic nature of this pesticide. Four serum hepatic marker enzymes (ALT, AST, LDH, and ALP) were evaluated for hepatotoxicity. According to the results, DIC caused a significant increase in the AST, ALT, LDH, and ALP activities in rats treated with DIC in comparison to those of controls. The reasons for such effect of DIC are not understood at present certainly. However, it is known that several of soluble enzymes of blood serum such as these enzymes have been considered as indicators of the hepatic dysfunction and damage. Also, the increase in the activities of AST and ALT in plasma of rats treated with DIC is mainly due to the leakage of these enzymes from the liver cytosol into the blood stream (Navarro et al., 1993). Further, ALT and AST levels are also of value indicating the existence of liver diseases, as this enzyme is present in large quantities in the liver. ALT increases in serum when cellular degeneration or destruction occurs in this organ (Hassoun and Stohs, 1995). On the other hand, phosphatases and dehydrogenases are important and critical enzymes in biological processes too. They are responsible for detoxification, metabolism, and biosynthesis of energetic macromolecules for different essential functions. Any interference in these enzymes leads to biochemical impairment and lesions of the tissue and cellular function (Khan et al., 2001). Yamaguchi et al. (1981) and Yousef et al. (2007) reported that the changes in the activities of these enzymes in $SnCl_2$-treated rats were regarded as the biochemical manifestation of the toxic action of inorganic tin. Also, Rahman et al. (2000) suggested that the increase in the activities of ALP and AcP in plasma might be due to the increased permeability of plasma membrane or cellular necrosis, and this showed the stress condition of the treated animals. Also, the increase in plasma LDH activity may be due to the

TABLE II. Effects of DIC on hematological constituents of rats

Hematological Constituent	Control (Mean ± SD)	5 ppm DID (Mean ± SD)	10 ppm DIC (Mean ± SD)
RBC ($\times 10^6$/mm^3)	8.7 ± 0.4	9.1 ± 0.1	9.3 ± 0.5
WBC ($\times 10^3$/mm^3)	5.3 ± 0.4	8.6 ± 0.7*	14.1 ± 1.5*
HGB (g/dL)	14.6 ± 1	15.5 ± 0.2	16.3 ± 0.7
HCT (%)	42.4 ± 2.8	45.4 ± 0.3	46.4 ± 2.2
MCV (μm^3)	50.2 ± 1.5	49.8 ± 0.4	49.9 ± 1.3
MCH (pg)	17.9 ± 0.5	21.1 ± 3.3	25.3 ± 4.2
MCHC (%)	34.5 ± 0.3	34.1 ± 0.5	35.1 ± 0.4
PLT ($\times 10^3$/mm^3)	553.2 ± 71	544.7 ± 34.1	540.8 ± 66.1

* Significantly different from control rats at $P \leq 0.05$ (One-way ANOVA).

hepatocellular necrosis leading to leakage of the enzyme to the blood stream (Wang and Zhai, 1988). So far, because no study examining the affects of DIC *in vivo* state have been made in rats, we did not have the chance to compare our results with the previous ones. However, Campbell and Ofurum (1986) demonstrated that short-term intoxication with DIC leads to increase of GOT and GPT in rats. Thus, these results are consistent with our results and support our results, although the treatment time and manner, the setting of studies and concentrations of the chemical are different.

In addition to the increased level of serum marker enzymes, the results of the present study have also demonstrated that the applied dosages of DIC could have promoted cell immune defense system in rats (Table II). It is clear from our observation that, upon DIC treatment *in vivo*, the numbers of WBC differ from those of controls. Namely, we observed that while DIC caused a significant increase in the level of WBC, the levels of the other hematological constituents did not change. From the observed values of WBC, it is clear that an increase in the number of WBC is a normal reaction of rats to the substance, which alters their normal physiological processes. The leucocytosis observed in present study indicates an immune system reaction to protect the rats against infection that might have been caused by chemical and also secondary infections, which may be contracted after the weakening of the condition of the rats. Leucocytosis, which may be directly proportional to the severity of the causative stress condition, may be attributed to an increase in leukocyte mobilization (Srivastava and Narain, 1985).

The observations presented led us to conclude that administrations of subacute DIC promote immune defense cells, elevate tissue damage serum marker enzymes, and deplete body weight in the rats. Thus, any external stressor, such as DIC, even at nonlethal concentration, can have a toxic effect on organism. From the foregoing observations, it is also postulated that hematological and serum enzymes might offer a certain result of choice for monitoring biotoxicity of direct acting compounds such as DIC. In addition, such a test will also be of value in pollution studies, and also be of interest to understand molecular basis of refractoriness of DIC toxicity. However, individual variations, in the biochemical and hematologic characters of animal, as proven in the past, are very important phenomena to consider when final conclusion is made.

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